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Characterization of moulds associated with processed garri stored for 40 days at ambient temperature in Makurdi, Nigeria

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Characterization of moulds associated with processed white and yellow garri stored at ambient temperature for 40 days was investigated. The moulds isolated from white garri (%) were: *Aspergillus* spp 35.3, *Penicillium* spp 23.53, *Fusarium* spp 2.94, *Mucor* spp 17.65, *Alternaria* spp 5.88, *Cladosporium* sp 2.94 and *Rhizopus* 11.76%. For yellow garri: *Aspergillus* spp 37.04, *Penicillium* spp 23.53, *Fusarium* spp 7.41, *Mucor* spp 18.52, *Rhizopus* spp 14.81, *Alternaria* spp 0% *Cladosporium* sp. and *Aspergillus* spp had the highest frequency of occurrence in both white and yellow garri. Higher moulds species were isolated from white garri (34) compared to yellow (27) samples. The mean total fungal counts from the three hostels were 6.22×10^3, 7.22×10^4 and 9.67×10^5 $CFUg^{-1}$ in white garri, and 3.56×10^3, 4.22×10^4 and 5.78×10^5 $CFUg^{-1}$ in yellow garri. There were significant differences in total mean fungal counts in the various dilutions of white and yellow garri at $p< 0.05$. Results also revealed that the longer the storage time, the higher the pH and moisture content. Proper storage is recommended owing to the public health concern due to mycotoxins, food safety, shelf life and biostability of this product.

Key words: Garri, moulds, storage, pH, moisture content.

INTRODUCTION

Garri is made from peeled, washed, grated, fermented and toasted fresh cassava tuber (*Manihot esculenta* Crantz). It is the most popular fermented cassava products in Africa (Oluwole et al., 2004; Ernesto et al., 2000). It is consumed by several millions of people in West Africa where it forms a major part of their diet (Edem et al., 2001; Kostinek et al., 2005; Ogiehor et al., 2007). In Nigeria, its acceptability cuts across the various ethnic and socio-economic classes, making it the commonest food item (Jekayinfa and Olajide, 2007; Ogiehor et al., 2007). Garri is stored and marketed in a ready-to-eat form and prepared into a stiff paste or dough-like called 'Eba' by adding the granules into hot water and stirring to make a paste of varied consistency. Eba can be consumed with local soups or stews of various types by chewing or swallowing in morsels (Asegbeloyin and Onyimonyi, 2007; Ogiehor et al., 2007). Garri can also be deliciously consumed directly (without cooking) with groundnut, smoked fish, coconut, cowpeas, moimoi, or taken as a fast food when soaked in cold water (Ogugbue and Obi, 2011). Sometimes, it is taken with beverages mixed with cold water or warm water with salt depending on the choice of the individual. Microbial growth, deterioration and spoilage of garri are major cause of food borne illnesses and threat to public health. However, some unhygienic practices involved in production, processing of cassava to garri and post processing handling such as spreading on the floor and mats after frying, displaying in

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open bowl or buckets in the markets during sales; the use of various packaging materials to transfer finished products from rural to urban areas and the use of bare hands during handling and sales may lead to microbial contamination due to deposition of bioaerosols on exposed products and transfer of infectious agent during handling (Ogiehor et al., 2007; Ogugbue and Obi, 2011; Ogugbue et al., 2011).

The main biological agents that contaminate and spoil garri are moulds, insects and mites (Igbeka, 1987; Ogiehor et al., 2005). Garri is rich in carbohydrate and therefore, suitable for fungal growth. Moulds such as *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizopus*, *Cladosporium* and *Mucor* have been associated with garri during storage and distribution (Ekundayo, 1984; Ogugbue et al., 2011). Several reports have revealed high occurrence of microorganisms in market samples of garri (Ijabadeniyi, 2007; Amadi and Adebola, 2008; Ogiehor et al., 2007). The growth of moulds in garri results in changes in the organoleptic, microbiological and nutritive quality which lead to spoilage of the food product (Efiuvwevwere and Isaiah, 1998). Some moulds such as *Aspergillus flavus*, *Aspergillus parasiticus* and *Penicillium* spp. can also produce aflatoxins (SubbaRao, 2000; Frazier and Westhoff, 2000; Ogiehor et al., 2007), which can have serious effects depending on the dosage consumed. Garri is a major food consumed among students in Africa, especially in Nigeria; hence, the present study was based on the need to assess the microbiological safety of garri consumed by these students in the University environment with respect to their storage conditions and packages used in storage.

MATERIALS AND METHODS

Sample collection

Two hundred (200) samples of garri (100 yellow and 100 white) were collected from three hostels within the University environment and transferred into clean factory sterilized polythene bags, labelled appropriately and transported to Microbiology Laboratory of the University for microbiological Examination. The samples were immediately analysed within 5 h of collection to avoid contamination from external source.

Moisture content and pH determination

Each sample was determined by a modification of method described by AOAC (1990). Briefly, the moisture content was determined by weighing 5.0 g each of all the samples and drying in an oven maintained at 105°C for 7 to 8 h for a constant weight to be obtained. Thereafter, they were stored in desiccators to cool and then re-weighed. The difference in weight was used to obtain the moisture content. Moisture content determination was done at every 5- day interval within 40 days. The pH of each garri samples were determined using a reference electrode pH meter by homogenizing 10 g of each garri sample in 10 ml of sterile distilled water and the pH of the sample determined (Ogiehor and Ikenebomeh, 2005).

Microbiological examination of garri samples

Sample preparation

The spread plate count method was used by weighing 10 g of each processed sample aseptically into 90 ml of 0.1% (w/v) sterile peptone water in a sterile 500 ml beaker, and allowed to stand for 5 min with occasional stirring using a magnetic stirrer as described by the method of Ogiehor and Ikenebomeh (2005). Furthermore, serial dilution method described by Jonathan and Olowolafe (2001) was carried out using 1 ml of initial suspension in the beaker in 9 ml of sterile distilled water in a 1:10 dilution. Further, serial dilutions were carried out using sterile pipettes to obtain up to 10^{-5} dilutions.

Isolation and identification of moulds

A 0.1 ml aliquot of each dilution of the suspension prepared earlier was pipetted and spread-plated in sterile Petri dish containing potato dextrose agar (PDA) supplemented with 0.1% concentrated lactic acid to inhibit bacterial growth. Inoculated plates were thereafter incubated at 28°C for 5 to 7 days. Total viable fungal counts were carried out and expressed as colony forming units per gram ($CFUg^{-1}$) of sample after incubation. Identification of the isolated moulds based on their morphological and cultural characteristics was carried out using the descriptions of Alexopoulus et al. (1996).

Statistical analyses

The various data obtained from this study were subjected to statistical analyses: ANOVA and student's t test to compare significant differences of means ($p< 0.05$) in the white and yellow garri using statistical software, SPSS version 17.0.

RESULTS

Changes in moisture content of both white and yellow garri samples are presented in Figure 1. The results indicate that there was slight increase in the moisture contents of both white and yellow garri sample at every 5-day interval within 40 days. The ranges of the moisture contents for the white and yellow garri were 7.6 to 12.5 and 6.3 to 11.7%, respectively. In the samples, the higher the storage, the higher the pH. The pH changes of both white and yellow garri samples are presented in Table 1. A lower pH range of 2.9 to 3.6 was recorded for yellow garri compared to 3.5 to 4.3 of white garri. However, both samples recorded a slight increase in pH value as the storage progressed. Generally, higher moulds species were isolated from white garri samples compared to yellow garri. The mean of total fungal counts from the three hostels were 6.22×10^3, 7.22×10^4 and 9.67×10^5 $CFUg^{-1}$ in white garri, and 3.56×10^3, 4.22×10^4 and 5.78×10^5 $CFUg^{-1}$ in yellow garri (Table 2). There were significant differences in total mean fungal counts in the various dilutions of white and yellow garri at $p< 0.05$.

A total of seven mould species (*Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., *Mucor* spp., *Alternaria* sp., *Cladosporium* sp and *Rhizopus* sp) were isolated from both white and yellow garri samples (Table 2). The prevalence of moulds in white garri were *Aspergillus* spp (35.3%), *Penicillium* spp (23.53%), *Fusarium* spp (2.94%), *Mucor* spp (17.65%), *Alternaria* sp (5.88%),

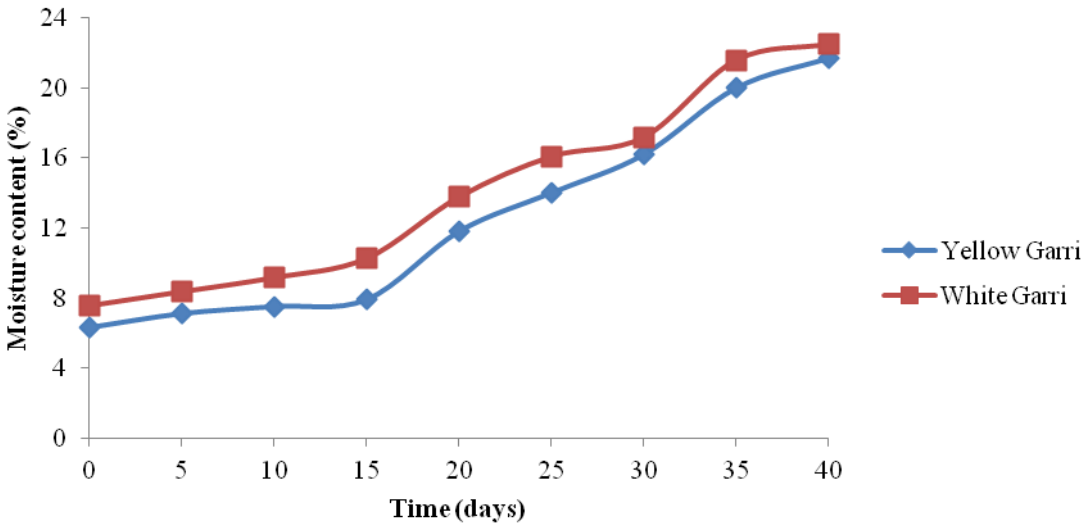

Figure 1. Changes in moisture content of the two types of garri stored at ambient temperature.

Table 1. Changes in pH of white and yellow garri stored at ambient temperature.

Time (day)	White garri	Yellow garri
0	4.3	3.6
5	4.3	3.6
10	4.3	3.3
15	4.3	3.1
20	3.9	3.1
25	3.9	3.1
30	3.5	3.1
35	3.5	2.9
40	3.5	2.9

Table 2. Changes in total viable fungal counts of white and yellow garri obtained from the university hostels during storage at ambient temperature.

Time (day)	Total fungal counts (TFC)					
	Hostel A ($CFUg^{-1} \times 10^3$)		Hostel B ($CFUg^{-1} \times 10^4$)		Hostel C ($CFUg^{-1} \times 10^5$)	
	White garri	Yellow garri	White garri	Yellow garri	White garri	Yellow garri
0	1	1	3	ND	7	3
5	3	1	7	1	9	3
10	6	2	3	4	8	4
15	5	4	8	2	11	5
20	7	4	9	9	9	9
25	7	4	6	2	11	6
30	5	6	7	6	9	4
35	11	4	13	6	10	9
40	11	6	9	8	13	9
Average TFC	6.22	3.56	7.22	4.22	9.67	5.78

ND, Not detected.

Cladosporium sp (2.94%) and *Rhizopus* (11.76%) while in yellow garri, the percentage occurrence were *Aspergillus* spp (37.04%), *Penicillium* spp (23.53%), *Fusarium* spp (7.41%), *Mucor* spp (18.52%) and *Rhizopus* (14.81%). How-

Table 3. Frequency of occurrence of mould species obtained from garri samples during the period of study.

Mould	Percentage (%) occurrence	
	Yellow garri	White garri
Aspergillus spp	10 (37.04)	12 (35.3)
Penicillium spp	6 (22.22)	8 (23.53)
Fusarium spp	2 (7.41)	1 (2.94)
Mucor spp	5 (18.52)	6 (17.65)
Alternaria sp	-	2 (5.88)
Cladosporium sp	-	1 (2.94)
Rhizopus sp	4 (14.81)	4 (11.76)
Total	27 (100)	34 (100)

However, *Alternaria* sp and *Cladosporium* sp were absent in yellow garri but present in white garri.

Aspergillus spp had the highest frequency of occurrence in both white (35.3%) and yellow (37.04%) garri from the overall isolates from the samples collected.

DISCUSSION

The percentage moisture contents of yellow garri (12.50%) and white garri (11.70%) supported the values of 12.7 to 13.6% previously reported (Halliday et al., 1967; Adeniyi, 1976). Ogugbue and Obi (2011) also reported higher moisture content in garri stored in different packages above the safe limit. In this study, it was observed that white garri had higher moisture content than yellow garri from day 0 to 40th day. The results of this study also reveal that the moisture content increased as the number of days increased and could eventually cause deterioration of the stored products. Halliday et al. (1967) reported that the major important factor that could encourage mould contamination and proliferation of garri is the high initial moisture content or increase in moisture content during storage.

The pH range of white garri (3.5 to 4.3) had higher values than yellow garri (2.9 to 3.6) at the end of the study. The pH of the yellow garri were within the pH range of 3.4 to 4.0 reported by Achinewhu et al. (1998) and white garri within the range of 4.19 to 4.58 reported by Owuamanam et al. (2011). The slight increase in pH obtained in both white and yellow garri may be due to production of acidic metabolites by microorganisms during their growth and proliferation (Ogugbue et al., 2011). The mould isolated from both yellow and white garri sample are *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., *Mucor* spp., *Alternaria* sp., *Cladosporium* sp and *Rhizopus* sp.

Obadina et al. (2009) also reported the isolation of similar moulds from their study on garri during assessment of some fermented cassava products. *Aspergillus* spp were the most predominant isolates in the present study. Several authors have isolated and identified number of mould species in garri under various storage conditions (Obadina et al., 2009; Ogugbue et al., 2011; Jonathan et al., 2011; Thomas et al., 2012). The mould contamination could have been introduced during local method of processing, exposure to bioaerosols during sale in the market and post processing handling of such product (Aboaba and Amisike, 1991; Okigbo, 2003; Ogeihor and Ikenebomeh, 2005; Amadi and Adebola, 2008; Ogubue et al., 2011). Samson et al. (2000) reported that almost all the common *Aspergillus* spp have been recovered at some time from agricultural products. The result of this study revealed that the longer the storage time, the higher the bio-load, pH and moisture content. Therefore, proper storage methods such as dry environment (with very low relative humidity and moisture) is required to prevent the growth and survival of moulds associated with garri and the public health concerns (such as food intoxication and illnesses) that may result from mycotoxins harbours by some of these fungi when they are consumed by the students.

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